

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Original) A polynucleotide comprising:
 - a) an amplifiable selectable gene;
 - b) a green fluorescent protein (GFP) gene; and
 - c) a selected sequence encoding a desired product, the selected sequence operably linked to either the amplifiable selectable gene or to the GFP gene, and to a promoter.
2. (Original) The polynucleotide of claim 1, wherein the amplifiable selectable gene is selected from the group of consisting of the genes encoding dihydrofolate reductase (DHFR) and glutamine synthetase.
3. (Original) The polynucleotide of claim 2, wherein the amplifiable selectable gene is the dihydrofolate reductase (DHFR) gene.
4. (Original) The polynucleotide of claim 1, wherein the GFP gene encodes a mutant GFP.
5. (Original) The polynucleotide of claim 4, wherein the mutant GFP exhibits a higher fluorescence intensity than the wild-type GFP.
6. (Original) The polynucleotide of claim 4, wherein the mutant GFP is GFP-S65T having a serine to threonine substitution in amino acid 65 of the wild-type GFP of *Aequorea victoria*.
7. (Original) The polynucleotide of claim 1, wherein the GFP gene encodes a GFP-fusion protein.

8. (Original) The polynucleotide of claim 1, wherein the amplifiable selectable gene is fused to the GFP gene as a fusion gene.
9. (Original) The polynucleotide of claim 8, wherein the amplifiable selectable gene is the DHFR gene.
10. (Original) The polynucleotide of claim 8, further comprising an intron between the promoter and the selected sequence, the intron defined by a 5' splice donor site and a 3' splice acceptor site.
11. (Original) The polynucleotide of claim 10, wherein the intron provides a splicing efficiency of at least 95%.
12. (Original) The polynucleotide of claim 10, wherein the fusion gene is positioned within the intron and wherein the fusion gene and selected sequence are operably linked to the promoter 5' of the intron.
13. (Original) The polynucleotide of claim 10, further comprising an internal ribosome entry site (IRES) between the selected sequence and the fusion gene, wherein the selected sequence and fusion gene are operably linked to the promoter 5' of the selected sequence.
14. (Original) The polynucleotide of claim 1, further comprising 3' of the promoter: an intron defined by a 5' splice donor site and a 3' splice acceptor site providing a splicing efficiency of at least 95%; and an IRES; wherein the selected sequence is positioned between the intron and the IRES.
15. (Original) The polynucleotide of claim 14, wherein the amplifiable selectable gene is positioned in the intron and the GFP gene is 3' of the IRES.
16. (Original) The polynucleotide of claim 14, wherein the GFP gene is positioned in the intron and the amplifiable selectable gene is 3' of the IRES.

17. (Original) The polynucleotide of claim 1, further comprising: a first transcription unit comprising a first promoter followed by an intron and the selected sequence; and a second transcription unit comprising a second promoter and an intron 3' of the second promoter; wherein the intron in the first transcription unit is the first intron, and the intron in the second transcription unit is the second intron, and wherein each of the first and the second introns is defined by a 5' splice donor site and a 3' splice acceptor site providing a splicing efficiency of at least 95%.

18. (Original) The polynucleotide of claim 17, wherein the amplifiable selectable gene is positioned in the intron in the first transcription unit wherein the amplifiable selectable gene and the selected sequence are both operably linked to the first promoter; and the GFP gene is positioned 3' of the second intron and operably linked to the second promoter in the second transcription unit.

19. (Original) The polynucleotide of claim 17, wherein the second transcription unit further comprises a selected sequence 3' of the second intron, wherein the selected sequence in the first transcription unit is the first selected sequence, and the selected sequence in the second transcription unit is the second selected sequence wherein the second selected sequence is operably linked to the second promoter and encodes a second desired product.

20. (Original) The polynucleotide of claim 19, wherein the amplifiable selectable gene is positioned in the first intron and operably linked to the first promoter, and the GFP gene is positioned in the second intron and operably linked to the second promoter.

21. (Original) The polynucleotide of claim 19, wherein the GFP gene is positioned in the first intron and operably linked to the first promoter, and the amplifiable selectable gene is positioned in the second intron and operably ~~linked~~ to the second promoter.

22. (Original) The polynucleotide of claim 19, further comprising an IRES 3' of the second selected sequence.

23. (Original) The polynucleotide of claim 22, wherein the amplifiable selectable gene is positioned in the first intron and operably linked to the first promoter, and the GFP gene is positioned 3' of the IRES and operably linked to the second promoter.
24. (Original) The polynucleotide of claim 19, wherein the amplifiable selectable gene is fused to the GFP gene to form a fusion gene wherein the fusion gene is positioned in the first intron.
25. (Original) The polynucleotide of claim 24, wherein the second transcription unit further comprises a selectable marker gene positioned in the second intron and operably linked to the second promoter.
26. (Original) The polynucleotide of claim 19, wherein the first transcription unit further comprises an IRES 3' of the first selected sequence.
27. (Original) The polynucleotide of claim 26, wherein the amplifiable selectable gene and the GFP gene are fused to form a fusion gene positioned 3' of the IRES and operably linked to the first promoter.
28. (Original) The polynucleotide of claim 27, wherein the second transcription unit further comprises a selectable marker gene positioned in the second intron and operably linked to the second promoter.
29. (Original) The polynucleotide of claim 26, wherein the second transcription unit further comprises an IRES 3' of the second selected sequence, wherein the IRES in the first transcription unit is the first IRES, and the IRES in the second transcription unit is the second IRES.
30. (Original) The polynucleotide of claim 29, wherein the amplifiable selectable gene is positioned 3' of the first IRES and operably linked to the first promoter, and GFP gene is positioned 3' of the second IRES and operably linked to the second promoter.

31. (Original) The polynucleotide of claim 19 wherein the first promoter and the second promoter are the same type of promoter.
32. (Original) The polynucleotide of claim 31, wherein the first promoter and the second promoter are the CMV or the SV40 promoter.
33. (Original) The polynucleotide of claim 19, wherein at least one of the promoters is inducible.
34. (Original) The polynucleotide of claim 1, wherein the promoter is the human cytomegalovirus immediate early (CMV) promoter.
35. (Original) The polynucleotide of claim 1, wherein the selected sequence encodes a protein selected from the group consisting of cytokines, lymphokines, enzymes, antibodies, and receptors.
36. (Original) The polynucleotide of claim 1, wherein the selected sequence encodes a protein selected from the group consisting of neuronotrophin-3, deoxyribonuclease, vascular endothelial growth factor, immunoglobulin and Her2 receptor.
37. (Original) The polynucleotide of claim 19, wherein the first selected sequence encodes an immunoglobulin heavy chain and the second selected sequence encodes an immunoglobulin light chain.
38. (Original) The polynucleotide of claim 19, wherein the first selected sequence encodes one polypeptide chain of a multichain receptor, and the second selected sequence encodes a second polypeptide chain of the receptor.
39. (Original) The polynucleotide of claim 1 that replicates in a eukaryotic host cell.
40. (Original) A polynucleotide comprising:
 - a) an amplifiable selectable gene;

- b) a fluorescent protein gene; and
- c) a selected sequence encoding a desired product, the selected sequence operably linked to the amplifiable selectable gene or to the fluorescent gene, and to a promoter.

41. (Original) A host cell comprising the polynucleotide of claim 1.

42. (Original) The host cell of claim 41, wherein the cell is a mammalian cell.

43. (Original) The host cell of claim 42 wherein the mammalian cell is a Chinese Hamster Ovary (CHO) cell.

44. (Original) The host cell of claim 43, wherein the amplifiable selectable gene is the DHFR gene and the CHO cell has a DHFR- phenotype.

45. (Original) The host cell of claim 43, wherein the desired product is selected from the group consisting of neuronotrophin-3, deoxyribonuclease, vascular endothelial growth factor, immunoglobulin and Her2 receptor.

46. (Original) A kit comprising a container containing the polynucleotide of claim 1.

47. (Original) A method of producing a desired product comprising introducing the polynucleotide of claim 1 into a suitable eukaryotic cell, culturing the resultant eukaryotic cell under conditions so as to express the desired product, and recovering the desired product.

48. (Original) The method of claim 47 wherein the desired product is recovered from the culture medium.

49. (Original) A method of obtaining a cell expressing a desired product, the method comprising:

- a) introducing the polynucleotide of claim 1 into a population of eukaryotic cells;
- and

b) isolating the cells of step a) that express the green fluorescent gene and the amplifiable selectable gene, expression indicative of the cell also expressing the desired product.

50. (Original) The method of claim 49, wherein the step of isolating cells expressing the green fluorescent protein gene comprises sorting for and cloning the brightest 1%-10% of fluorescent cells, wherein the sorting and cloning are performed using a fluorescence activated cell sorter.

51. (Original) The method of claim 50, wherein the cells are subjected to two or more rounds of sorting, wherein the cells are cultured for a period of time between each round.

52. (Original) The method of claim 51, wherein the cells are cultured for about two weeks between each round of sorting.

53. (Original) The method of claim 52, wherein the cells are cultured in selection medium.

54. (Original) The method of claim 52, wherein the brightest fluorescent cells are cultured in selection medium comprising an appropriate amplifying agent.

55. (Original) The method of claim 52, wherein the brightest fluorescent cells are cultured in medium containing incremental amounts of the amplifying agent.

56. (Original) The method of claim 53, wherein the amplifiable selectable gene is DHFR and the amplifying agent is methotrexate.

57. (Original) The method of claim 54, further comprising analyzing the cells after culture with amplifying agent, for expression of the desired product to isolate cells producing high levels of the desired product.

58. (Original) The method of claim 57, wherein the cells are analyzed for RNA encoding the desired product by RT-PCR, the amount of RNA indicative of the level of production of the desired product.

59. (New) A polynucleotide comprising:
a fusion gene comprising a first selectable gene and an amplifiable second selectable gene; and
a selected sequence encoding a desired product, the selected sequence operably linked to the amplifiable selectable gene and to a promoter.

60. (New) The polynucleotide of claim 59, wherein the amplifiable second selectable gene is selected from the group of consisting of the genes encoding dihydrofolate reductase (DHFR) and glutamine synthetase.

61. (New) The polynucleotide of claim 60, wherein the amplifiable selectable gene is the dihydrofolate reductase (DHFR) gene.

62. (New) The polynucleotide of claim 61, wherein the first selectable gene of the fusion gene is not amplifiable.

63. (New) The polynucleotide of claim 62, wherein the first selectable gene of the fusion is selectable independent of the amplifiable selectable gene.

64. (New) The polynucleotide of claim 62, wherein the first selectable gene is an antibiotic resistance gene.

65. (New) The polynucleotide of claim 64, wherein the first selectable gene is a gene encoding puromycin resistance.

66. (New) The polynucleotide of claim 59, wherein the fusion gene comprises an antibiotic resistance gene fused to the DHFR gene.

67. (New) The polynucleotide of claim 59, wherein the fusion gene is positioned within an intron between the promoter and the selected sequence, the intron defined by a 5' splice donor site comprising a splice donor sequence and a 3' splice acceptor site.

68. (New) The polynucleotide of claim 67, wherein the efficiency of splicing a messenger RNA having the splice donor sequence is between about 80% and 99% as determined by quantitative PCR.

69. (New) The polynucleotide of claim 68, wherein the intron provides a splicing efficiency of at least 95%.

70. (New) The polynucleotide of claim 67, wherein the fusion gene is positioned within the intron and wherein the fusion gene and selected sequence are operably linked to the promoter 5' of the intron.

71. (New) The polynucleotide of claim 67, further comprising an internal ribosome entry site (IRES) between the selected sequence and the fusion gene, wherein the selected sequence and fusion gene are operably linked to the promoter 5' of the selected sequence.

72. (New) The polynucleotide of claim 59, further comprising one or more additional selected sequences encoding a desired product, the one or more additional selected sequences operably linked to an amplifiable selectable gene and to a promoter.

73. (New) The polynucleotide of claim 72, wherein the first selected sequence encoding a first desired product is operably linked to a first promoter and a second selected sequence encoding a second desired product is linked to a second promoter.

74. (New) The polynucleotide of claim 73, wherein the first and second promoters are the same type of promoter.

75. (New) The polynucleotide of claim 74, wherein the first and second promoters are from SV40.

76. (New) The polynucleotide of claim 74, wherein the first and second promoters are from CMV.
77. (New) The polynucleotide of claim 73, wherein at least one of the promoters is inducible.
78. (New) The polynucleotide of claim 77, wherein each of the promoters is inducible.
79. (New) The polynucleotide of claim 74, wherein the promoter is the human cytomegalovirus immediate early (CMV) promoter.
80. (New) The polynucleotide of claim 59, wherein the selected sequence encodes a protein selected from the group consisting of cytokines, lymphokines, enzymes, antibodies, and receptors.
81. (New) The polynucleotide of claim 80, wherein the selected sequence encodes a protein selected from the group consisting of neuronotrophin-3, deoxyribonuclease, vascular endothelial growth factor, immunoglobulin and Her2 receptor.
82. (New) The polynucleotide of claim 73, wherein the first selected sequence encodes an immunoglobulin heavy chain and the second selected sequence encodes an immunoglobulin light chain.
83. (New) The polynucleotide of claim 73, wherein the first selected sequence encodes one polypeptide chain of a multichain receptor, and the second selected sequence encodes a second polypeptide chain of the receptor.
84. (New) The polynucleotide of claim 59 that replicates in a eukaryotic host cell.
85. (New) A host cell comprising the polynucleotide of claim 59.

86. (New) The host cell of claim 85, wherein the cell is a mammalian cell.
87. (New) The host cell of claim 86 wherein the mammalian cell is a Chinese Hamster Ovary (CHO) cell.
88. (New) The host cell of claim 87, wherein the amplifiable selectable gene is the DHFR gene and the CHO cell has a DHFR- phenotype.
89. (New) The host cell of claim 86, wherein the desired product is selected from the group consisting of neuronotrophin-3, deoxyribonuclease, vascular endothelial growth factor, immunoglobulin and Her2 receptor.
90. (New) A kit comprising a container containing the polynucleotide of claim 59.
91. (New) A method of producing a desired product comprising introducing the polynucleotide of claim 59 into a suitable eukaryotic cell, culturing the resultant eukaryotic cell under conditions so as to select and amplify the fusion gene and selected gene encoding the desired product, expressing the desired product, and recovering the desired product.
92. (New) The method of claim 91 wherein the desired product is recovered from the culture medium.
93. (New) The polynucleotide of claim 72 that replicates in a eukaryotic host cell.
94. (New) A host cell comprising the polynucleotide of claim 72.
95. (New) The host cell of claim 94, wherein the cell is a mammalian cell.
96. (New) The host cell of claim 95 wherein the mammalian cell is a Chinese Hamster Ovary (CHO) cell.

97. (New) The host cell of claim 96, wherein the amplifiable selectable gene is the DHFR gene and the CHO cell has a DHFR- phenotype.
98. (New) The host cell of claim 95, wherein the desired product is selected from the group consisting of neuronotrophin-3, deoxyribonuclease, vascular endothelial growth factor, immunoglobulin and Her2 receptor.
99. (New) A kit comprising a container containing the polynucleotide of claim 72.
100. (New) A method of producing a desired product comprising introducing the polynucleotide of claim 72 into a suitable eukaryotic cell, culturing the resultant eukaryotic cell under conditions so as to select and amplify the fusion gene and selected gene encoding the desired product, expressing the desired product, and recovering the desired product.
101. (New) The method of claim 100 wherein the desired product is recovered from the culture medium.